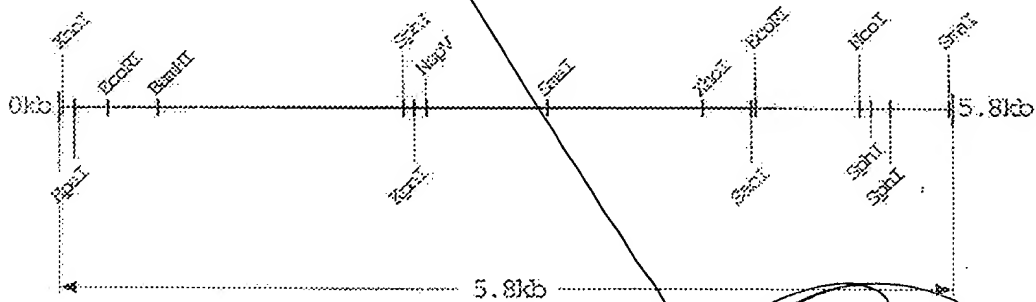


Sub
F1

restriction site, 2 EcoRI restriction sites, 1 HpaI
restriction site, 1 KpnI restriction site, 1 NcoI restriction
site, 1 NspV restriction site, 1 SacI restriction site, 2 SmaI
restriction sites, 3 SphI restriction sites, 2 XhoI
restriction sites, no ClaI restriction site, no DraI
restriction site, no EcoRV restriction site, no HindIII
restriction site, no NdeI restriction site, no NheI
restriction site, no PvuII restriction site, no ScaI
restriction site, no Sse8387I restriction site, no StuI
restriction site, and no XbaI restriction site, and having a
restriction map of:



C1
cont.

, said isolated DNA-fragment derived from Borkholderia cepacia
KK01.

Sub
D3

2. (Amended) The isolated DNA fragment, said
isolated DNA-fragment derived from Borkholderia cepacia KK01

C1 wherein the DNA fragment has a nucleotide sequence of SEQ ID NO: 1 in the Sequence Listing.

Sub D3
3. (Amended) An isolated DNA fragment having a nucleotide sequence of SEQ ID NO: 1 with substitution, with substitution of at least one nucleotide, said substitution resulting in 1) no amino acid change with code degeneration, or 2) amino acid substitution only between aliphatic amino acids, between sulfur-containing amino acids, between hydroxy amino acids, between aromatic amino acids, between basic amino acids, and between acidic amino acids.

5. (Amended) The recombinant DNA fragment according to claim 4, wherein the vector can be maintained or replicated in a bacterium.

Sub E3
CP2
6. (Amended) An isolated DNA fragment containing a region encoding a toluene monooxygenase, the region comprising a first sequence encoding a polypeptide TomL having an amino acid sequence of SEQ ID NO: 3, a second sequence encoding a polypeptide TomM having an amino acid sequence of SEQ ID NO: 4, a third sequence encoding a polypeptide TomN

Sub F-3
having an amino acid sequence of SEQ ID NO: 5, a fourth sequence encoding a polypeptide TomO having an amino acid sequence of SEQ ID NO: 6, and a fifth sequence encoding a polypeptide TomP having an amino acid sequence of SEQ ID NO: 7 of the Sequence Listing, and the first to fifth sequences are aligned so that expressed TomL - TomP polypeptides can form an active ^{toluene} monooxygenase protein.

Sub F-3
7. (Amended) An isolated DNA fragment according to claim 6, wherein no spacer sequence is present between the first to fifth sequences or at least one spacer sequence is present between the first to fifth sequences.

C2 Cont Sub F-3
8. (Amended) An isolated DNA fragment according to claim 6 or 7, further comprising a sequence encoding a polypeptide TomQ having an amino acid sequence of SEQ ID NO: 8 in the Sequence Listing.

Sub F-3
9. (Amended) An isolated DNA fragment containing a region encoding a toluene monooxygenase, wherein the region comprises a first sequence encoding a polypeptide TomL having an amino acid sequence of SEQ ID NO: 3, a second sequence

Seq 4
encoding a polypeptide TomM having an amino acid sequence of SEQ ID NO: 4, a third sequence encoding a polypeptide TomN having an amino acid sequence of SEQ ID NO: 5, a fourth sequence encoding a polypeptide TomO having an amino acid sequence of SEQ ID NO: 6, and a fifth sequence encoding a polypeptide TomP having an amino acid sequence of SEQ ID NO: 7, and the first to fifth sequences are aligned so that expressed TomL - TomP polypeptides can form an active monooxygenase protein;

C2 cont.
wherein in at least one of the first to fifth sequences of the DNA fragment substitution with substitution of at least one nucleotide, said substitution resulting in 1) no amino acid change with code degeneration, or 2) amino acid substitution only between aliphatic amino acids, between sulfur-containing amino acids, between hydroxy amino acids, between aromatic amino acids, between basic amino acids, and between acidic amino acids.

10. (Amended) An isolated DNA fragment comprising a region encoding a polypeptide TomK, the polypeptide TomK having an amino acid sequence of SEQ ID NO: 2, with substitution of at least one nucleotide, said substitution

resulting in 1) no amino acid change with code degeneration,
or 2) amino acid substitution only between aliphatic amino
acids, between sulfur-containing amino acids, between hydroxy
amino acids, between aromatic amino acids, between basic amino
acids, and between acidic amino acids.

Sub
D4

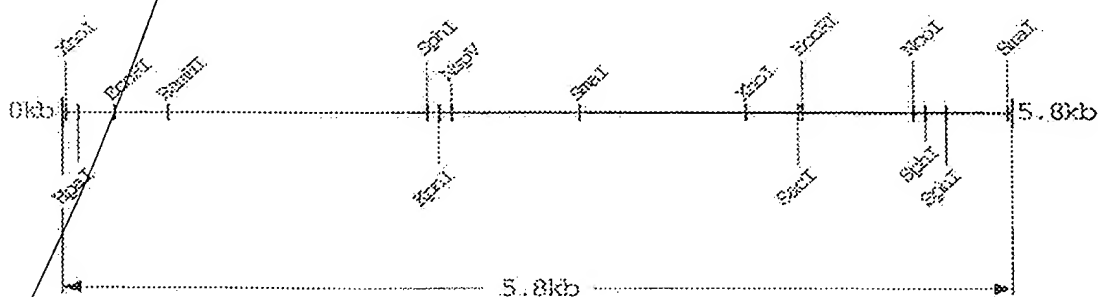
11. (Twice Amended) A recombinant DNA comprising a
vector, a promoter, and the DNA fragment according to any one
of claims 6, 7 or 9, wherein the vector and the promoter are
functionally ligated to the DNA fragment to enable expression
of the toluene monooxygenase encoded by the DNA fragment.

C2
Cont

15. (Amended) A transformant obtained by
introducing a recombinant DNA into a host microorganism, the
recombinant DNA comprising a vector enabling maintenance or
replication in a host and a DNA fragment of about 5.8 Kb
containing a toluene monooxygenase gene, having 1 BamHI
restriction site, 2 EcoRI restriction sites, 1 HpaI
restriction site, 1 KpnI restriction site, 1 NcoI restriction
site, 1 NspV restriction site, 1 SacI restriction site, 2 SmaI
restriction sites, 3 SphI restriction sites, 2 XhoI
restriction sites, no ClaI restriction site, no DraI

C3
Sub
D3

restriction site, no EcoRV restriction site, no HindIII
 restriction site, no NdeI restriction site, no NheI
 restriction site, no PvuII restriction site, no ScaI
 restriction site, no Sse8387I restriction site, no StuI
 restriction site, and no XbaI restriction site, and having a
 restriction map of DNA fragment of about 5.8 Kb containing a
 toluene monooxygenase gene having 1 BamHI restriction site, 2
 EcoRI restriction sites, 1 HpaI restriction site, 1 KpnI
 restriction site, 1 NcoI restriction site, 1 NspV restriction
 site, 1 SacI restriction site, 2 SmaI restriction sites, 3
 SphI restriction sites, 2 XhoI restriction sites, no ClaI
 restriction site, no DraI restriction site, no EcoRV
 restriction site, no HindIII restriction site, no NdeI
 restriction site, no NheI restriction site, no PvuII
 restriction site, no ScaI restriction site, no Sse8387I
 restriction site, no StuI restriction site, and no XbaI
 restriction site, and having a restriction map of:



Q3 sub
cont D5
said DNA-fragment derived from Borkholderia cepacia KK01.

17. (Amended) A transformant obtained by
introducing a recombinant DNA into a host microorganism, where
the recombinant DNA comprises a vector enabling maintenance or
replication in a host, and a DNA fragment ligated thereto
having a nucleotide sequence of SEQ ID NO: 1 of the Sequence
Listing with deletion, substitution and/or addition of one or
more nucleotides, still encoding an active toluene
monooxygenase, wherein the DNA fragment has a toluene
monooxygenase region of 4.9 kb or less.

55. (Amended) A recombinant DNA comprising a
vector, a promoter, a first DNA fragment being the DNA
fragment of any one of claims 6, 7 or 9, and a second DNA
fragment, said second DNA fragment comprising a region
encoding a polypeptide TomK having an amino acid sequence of
SEQ ID NO: 2, and a property to enhance the toluene
monooxygenase activity of a protein comprised of at least TomL
to TomP; or a region encoding a variety of TomK in which the
amino acid sequence of SEG ID NO. 2 is altered with the
proviso that the property to enhance the toluene monooxygenase